REMARKS

Claims 1-4, 11, 13, 14 and 22 are pending in the application. Claims 4 and 22 are allowed and claims 1-3, 11, 13 and 14 are currently rejected.

I. Request for Interview

Applicants respectfully request a personal telephonic interview with the Examiner prior to the issuance of the first Office Action. The purpose of the interview is to discuss the Examiner's position concerning the scope of the claims of the present invention.

The Examiner is requested to contact the undersigned Applicant's representative to arrange a date for an interview.

II. Amendments to the Claims

The claims have been amended to more fully comply with U.S. format and to more clearly state that which Applicants regard as their invention. In addition, the following specific amendments have been made.

Claims 1 and 11 have been amended to recite polypeptides with an approximate molecular weight of about 47 kDa as determined by SDS-PAGE and about 51 kDa as determined by sequence-based computer algorithm. This amendment is supported at page 47, lines 8-10 of the specification, by the executed Declaration Under 37 C.F.R. § 1.132 by Shigeru Yamamoto filed herewith, and by the executed Declaration Under 37 C.F.R. § 1.132 by Mr. Yamamoto filed October 17, 2003.

Claim 2 has been amended to correct the spelling of the disaccharide glycoside "apiofuranosyl," which was previously misspelled as "aviofuranosyl" due to a typographical error. A corresponding typographical error has also been corrected at page 76 of the specification (see Amendments to the Specification above).

III. Specification Objection

At page 3 of the Office Action, the Examiner objected to the improper denotation of the trademarks on page 26 of the specification at lines 17, 18, 20, 21 and 25.

Accordingly, Applicants have amended the listed trademarks on page 26 of the specification so that they are accompanied by the generic terminology, as required by the Examiner.

Applicants respectfully request reconsideration and withdrawal of this objection.

IV. Claim Rejections Under 35 USC § 101

At paragraph 7 on page 3 of the Office Action, claim 3 was rejected under 35 U.S.C. § 101 as being directed to non-statutory subject matter.

Specifically, the Examiner stated that the claim does not recite isolated or purified products, and therefore recites non-patentable products of nature.

In response, Applicants have amended claim 3 to recite "isolated" polypeptide variants. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

V. Claim Rejections Under 35 USC § 112, Second Paragraph - Indefiniteness

At paragraph 9 on page 3 of the Office Action, claims 1, 2, 11, 13, and 14 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention.

The Examiner explained that claims 1 and 11 are confusing, because the claims recite a single polypeptide isolated from a plurality of microorganisms.

Applicants have amended claims 1 and 11 to recite polypeptides isolated from a microorganism "selected from the group consisting of . . ." as suggested by the Examiner, and therefore respectfully request reconsideration and withdrawal of this rejection.

VI. Claim Rejections Under 35 USC § 112, First Paragraph - New Matter

At paragraph 10 on page 4 of the Office Action, claims 1, 2, 11, 13, and 14 were rejected under 35 USC § 112, first paragraph, as containing subject which was not described in the specification. The Examiner indicated that this is a new matter rejection.

The Examiner explained that the new matter rejection is maintained due to newly added limitations to the claims. Specifically, the Examiner contended that there is no support in the specification for a polypeptide having a molecular weight (MW) in the range of 47-51 kDa, for culture conditions in a pH range of 5-6, or for the glycosides rutinose and apiofuranosyl.

A. Molecular Weight

While the Examiner acknowledged support in the specification for a polypeptide having a MW of 47 kDa, and for a polypeptide having a MW of 51 kDa, the Examiner contended that there is not support for a polypeptide having a MW in the range of 47 - 51 kDa.

As noted above, claims 1 and 11 have been amended to recite polypeptides with "an approximate molecular weight of about 47 kDa as determined by SDS-PAGE and about 51 kDa as determined by sequence-based computer algorithm."

Polypeptides of the present invention with an approximate molecular weight of about 47 kDa as determined by SDS-PAGE are disclosed, for example, at page 47, lines 8-10 of the specification. Specifically, page 47 describes the results of an SDS-PAGE analysis of diglycosidase polypeptide derived from *Aspergillus fumigatus*, confirming that the enzyme can be purified as a single band of about 47 kDa. Furthermore, as described in the executed Declaration Under 37 C.F.R. § 1.132 by Mr. Yamamoto, filed October 17, 2003, the approximate molecular weight of the same polypeptide by computer algorithm based on amino acid sequence is about 51 kDa.

In view of the amendments to the claims and the foregoing comments, Applicants assert that there is support in the specification for a polypeptide having the approximate molecular weight recited in claims 1 and 11, and therefore respectfully request reconsideration and withdrawal of this rejection.

B. pH

The Examiner stated that there is no support in the specification, claims, or drawings as originally filed for culture conditions in a pH ranging from 5 - 6.

In response, Applicants respectfully submit that the specification provides support, on page 14, lines 13 and 14, for culture conditions in a pH ranging from 5 - 6. Specifically, the specification describes producing diglycosidase by culturing a microorganism in liquid medium wherein "the . . . pH is adjusted to a level of approximately from 3 to 8, more preferably from about 5 to 6."

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

C. Glycosides

The Examiner stated that he can find no support in the specification, claims, or drawings as originally filed for rutinose and "aviofuranosyl" (actually "apiofuranosyl," see section I. above), two of the glycosides recited in claim 2.

Applicants respectfully direct the Examiner's attention to Example 6, on page 76 of the specification. Example 6 describes the hydrolysis of various glycosides, including rutinose and apiofuranosyl, by diglycosidase preparations derived from various microorganisms.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

VII. Claim Rejections Under 35 U.S.C. § 112, First Paragraph - Written Description

At paragraph 11 on page 5 of the Office Action, claims 1-3, 11, 13, and 14 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

The rejected claims are drawn to a genus of polypeptides having the following characteristics: i) the ability to release saccharides from a disaccharide glycoside in a disaccharide unit, wherein the disaccharide glycoside has a glucose moiety at the aglycon side; ii) enzymatic activity at pH 2.5-3; iii) stable at 50°C or less; iv) approximate MW of about 47 kDa as determined by SDS-PAGE and about 51 kDa as determined by sequence-based computer algorithm; and v) isolated from the genus *Aspergillus, Penicillium, Rhizopus, Rhizomucor, Talaromyces, Mortierella, Cryptococcus, Microbacterium, Corynebacterium,* and *Actinoplanes.* The claims also recite a method for producing the polypeptides, and variants of the polypeptides.

A. Claims 1, 2, 11, 13, and 14

The Examiner contended that the specification fails to adequately describe the entire genus claimed. According to the Examiner, the specification discloses only a single representative species of the genus of claimed polypeptides, namely the polypeptide of SEQ ID NO: 8 isolated from *Aspergillus fumigatus.* The specification allegedly fails to describe any additional representative species of the claimed genus.

The Examiner acknowledged that the specification discloses diglycosidase production by individual species of the genera of microorganisms recited in the claims. The Examiner also noted that the specification further describes physicochemical characteristics of diglycosidase from these microorganisms. However, the Examiner concluded that because there is no evidence of record suggesting the recited microorganisms produce diglycosidase similar in MW to *Aspergillus fumigatus* diglycosidase of SEQ ID NO:8, the relevant identifying characteristics of SEQ ID NO: 8 are insufficient to describe the entire genus.

The Examiner also contended that the single disclosed species (i.e. SEQ ID NO:8) fails to represent the entire genus claimed, because the claimed genus encompasses species having variant functions. The Examiner pointed out that the function recited in claim 1 is the ability to release saccharides from a disaccharide glycoside in a disaccharide unit, wherein said disaccharide glycoside has a glucose moiety at the aglycon side. However, according to the Examiner, although the specification indicates that the *Aspergillus fumigatus* diglycosidase possesses β-primeverosidase activity, "the specification provides no indication that the disclosed polypeptide has the ability to hydrolyze *any* disaccharide to release a saccharide."

Applicants respectfully traverse this rejection, and submit that the claimed invention is adequately described in the specification.

With regard to the Examiner's first contention, Applicants note that the Declaration evidence of Mr. Katzutaka Tsuruhami, submitted herewith, suggests that the other microorganisms of the invention produce diglycosidase enzymes similar in MW to *Aspergillus fumigatus* diglycosidase of SEQ ID NO: 8. Specifically, Mr. Tsuruhami has shown that the diglycosidase derived from *Penicillium multicolor* has an approximate MW of about 49.9 kDa to 53.5 kDa as determined by SDS-PAGE. Applicants note that the diglycosidase derived from *Penicillium multicolor* also has the same enzymological properties as the diglycosidase from *Aspergillus fumigatus* (see Tsuruhami Declaration).

Furthermore, the hybridization results described at Example 10 on page 70 of the specification would indicate to a person of ordinary skill in the art that the diglycosidases of other microorganisms recited in the claims are structurally similar to the enzyme isolated from

Aspergillus fumigatus. Specifically, the results on page 70 show that the diglycosidase gene from *Aspergillus fumigatus* hybridizes under highly stringent conditions (hybridization at 5 x SSC, 0.1% N-lauroylsarcosine sodium, 0.02% SDS, 65°C, overnight; followed by washing at 6 x SSC, 0.1% SDS, room temperature, 5 min. x 2 and 6 x SSC, 0.1% SDS, 45°C, 15 min. x 2) to chromosomal DNA from all microorganisms demonstrating diglycosidase activity. Furthermore, signal was detected in *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus aculeatus*, *Penicillium multicolar*, *Penicillium lilacinum*, *Corynebacterium ammonia*genes and *Corynebacterium glutamicum*, at even under more stringent washing conditions (5 x SSC, room temperature, 10 min. and 4 x SSC, 65°C, 30 min.).

Because highly stringent hybridization conditions yield structurally similar genes, a person of ordinary skill in the art would not expect substantial variation between the diglycosidase gene of *Aspergillus fumigatus* and the hybridizing genes of the other microorganisms. It follows that a person of ordinary skill in the art would also recognize that polypeptides encoded by the hybridizing genes would be structurally similar to the polypeptide encoded by the *Aspergillus fumigatus* gene.

With regard to the Examiner's second contention, Applicants submit that a person of ordinary skill in the art would expect that the substrate specificity of *Aspergillus fumigatus* diglycosidase is the same or similar to that of the enzyme isolated from the other recited sources.

First, the diglycosidase enzymes from *Aspergillus fumigatus* (Examples 2 and 4 on pages 32 and 34 of the specification, respectively) and from the other microorganisms listed in claim 1 (Example 5, on page 35) have all been shown to demonstrate primeverosidase activity. In

addition, the specification discloses that diglycosidase from the various microorganisms listed in Example 5 acts on rutinose, gentibiose, arabinofuranosyl, and apiofuranosyl as well as on primeveroside glycosides. Furthermore, although the specification does not appear to describe an equivalent substrate specificity for *Aspergillus fumigatus* glycosidase, *Aspergillus oryzae, niger,* and *aculeatus* are among the strains listed in Table 6 (page 45) as possessing broad substrate specificity. Thus, one of ordinary skill in the art would also expect the diglycosidase from *Aspergillus fumigatus* to demonstrate the same activity. Finally, because the enzymes of the various microorganisms listed in claim 1 all act on rutinose, gentibiose, arabinofuranosyl, apiofuranosyl, and primeveroside glycosides (see Example 16 on page 76), one of ordinary skill would expect the claimed enzyme to act on *any* disaccharide glycoside that has a glucose moiety at the aglycon side.

In sum, because the Declaration evidence as well as the hybridization results demonstrate that the diglycosidases are structurally similar, and the enzyme activity assays demonstrate functional equivalence, a person of ordinary skill in the art would consider Applicants to be in possession of the genus of enzymes encompassed within the scope of claim 1.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

B. Claim 3

Claim 3 recites variant polypeptides that are at least 95% homologous to SEQ ID NO:8 and maintain the function of the natural enzyme. Applicants submit that the variants of claim 3 are adequately described. First, a person of ordinary skill in the art would expect highly

homologous variants of SEQ ID NO: 8 to have the same functional activity as the natural protein. In addition, procedures for making proteins with deletions, additions, insertions, and substitutions are routine in the art, and are described in the specification at pages 24-27. Finally, the specification provides at least one assay for identifying other proteins having the claimed enzyme activity, at pages 5-6.

Thus, the present specification indicates that the genus of proteins that must be variants of SEQ ID NO: 8 does not have substantial variation, since all of the variants must possess the specified enzymatic activity and must have at least 95% homology to SEQ ID NO: 8. The single species disclosed is representative of the genus because all members have at least 95% structural identity with SEQ ID NO: 8 and because of the disclosure of an assay, provided in the specification, for identifying all of the at least 95% identical variants of SEQ ID NO: 8 which are capable of the claimed activity. One of ordinary skill in the art would conclude that Applicants were in possession of the necessary common attributes possessed by the members of the genus. Please see *PTO Revised Written Description Guidelines Training Materials*, Example 14.

In view of the foregoing arguments, Applicants respectfully request reconsideration and withdrawal of this rejection.

VIII. Claim Rejections Under 35 U.S.C. § 112, First Paragraph - Enablement

At paragraph 12 on page 8 of the Office Action, claims 1-3, 11, 13 and 14 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement.

A. Claims 1, 2, 11, 13, and 14

The Examiner contends that the specification, while being enabling for the polypeptide of SEQ ID NO:8 and a method for making same, does not reasonably provide enablement for all polypeptides having the recited characteristics isolated from any microorganism encompassed in the recited genera.

Specifically, the Examiner states that the invention claimed (1) is overly broad in scope, because while the claims encompass polypeptides having a variety of enzymatic activities, the disclosure is limited to a polypeptide from *Aspergillus fumigatus* with β-primeverosidase activity; (2) is lacking in guidance, because the specification provides only a single working example of a polypeptide which meets the criteria set forth in the claims; (3) is highly unpredictable, because the purification scheme described is dependent upon the amino acid composition and MW of the polypeptide of SEQ ID NO:8, and may not apply to other diglycosidases; and (4) requires undue experimentation, because while methods of isolating a polypeptide are known, it is not routine in the art to devise purification schemes for all polypeptides having the recited structures, activities, and MWs isolated from all other organisms as encompassed by the instant claims.

Applicants respectfully traverse this rejection, and submit that the specification does provide enablement for the polypeptides isolated from organisms other than *Aspergillus fumigatus.*

First, the recited function does not lead to claims that are overly broad in scope, because the diglycosidase from *Aspergillus fumigatus* as well as from the other microorganisms listed in

claim 1 has been shown to act on any disaccharide glycoside that has a glucose moiety at the aglycon side (see above).

Second, the specification provides sufficient guidance for making and using the scope of claimed polypeptides. As noted above, the hybridization results described in Example 10 of the specification indicate that the diglycosidases are highly conserved. Thus, a person of ordinary skill in the art would expect that the structurally similar diglycosidases could be easily isolated, given the sequence of the diglycosidase of *Aspergillus fumigatus*. For example, the specification at page 21 describes a method of obtaining the diglycosidase gene from genomic or cDNA libraries of other microorganisms, using the gene from *Aspergillus fumigatus* as a probe for hybridization. In addition, a routine PCR-based method of obtaining the gene is described on page 22. Furthermore, a person of ordinary skill in the art would predict that the protein purification scheme outlined on pages 45 to 47 would apply to other diglycosidases as well as the enzyme of *Aspergillus fumigatus*. In fact, at page 2 of the Declaration submitted herewith, Mr. Tsuruhami has shown that the diglycosidase from *Penicillium multicolor* can be purified in accordance with the disclosed protein purification method.

Finally, because of the structural as well as the functional similarity of the various diglycosidases encompassed by the claims, and the guidance provided in the specification, making and using the recited invention would not require undue experimentation.

Therefore, Applicants respectfully request reconsideration and withdrawal of this rejection.

B. Claim 3

At page 11 of the Office Action, the Examiner contends that the variants recited in claim 3 are not enabled, because the positions within an amino acid sequence where modification can be made with a reasonable expectation of success in obtaining a variant having the desired activity are limited, and the result of such modifications is highly unpredictable.

In response, Applicants respectfully submit that claim 3 is fully enabled as well as described by the specification. The specification discloses methods for synthesizing variants, and methods for obtaining variants were also well known in the art at the time the application was filed. Furthermore, Applicants have provided assays for detecting the function of such variants. Due to the structural similarity of the members of the genus recited in claim 3 (all variants must have at least 95% homology to SEQ ID NO: 8), one of ordinary skill in the art would expect that a substantial number of variants would possess the claimed enzymatic activity. Thus, although a person of ordinary skill in the art would not necessarily be able to predict which particular amino acid modifications would lead to a functional polypeptide, mutagenesis would be likely to result in at least some functional polypeptides.

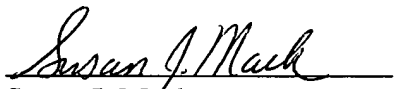
Furthermore, the experimentation necessary to synthesize and screen the variant polypeptides would be merely routine, and would not be undue. Practitioners in this art would be prepared to screen negative variants in order to find polypeptides that have the desired activity. Thus, even an arguably low success rate would not demonstrate a high level of unpredictability or unreliability in the art.

IX. Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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